models due to the severe autoimmune phenotype observed in mice containing T cells engineered to conditionally lack TGFBR2. Consequently, a need exists for effective strategies to reduce or eliminate the T cell inhibitory impact of TGF-β, including in the context of T cell mediated immunotherapy.

[0006] CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) evolved in bacteria and archea as an adaptive immune system to defend against viral attack. Upon exposure to a virus, short segments of viral DNA are integrated into the CRISPR locus. RNA is transcribed from a portion of the CRISPR locus that includes the viral sequence. That RNA, which contains sequence complementary to the viral genome, mediates targeting of an RNA-guided nuclease to a target sequence in the viral genome. The RNA-guided nuclease, in turn, cleaves and thereby silences the viral target.

[0007] Recently, the CRISPR/Cas9 system has been adapted for genome editing in eukaryotic cells. The introduction of site-specific double strand breaks (DSBs) allows for target sequence alteration through endogenous DNA repair mechanisms, for example non-homologous end-joining (NHEJ) or homology-directed repair (HDR). CRISPR/Cas9 represents a promising avenue for addressing TGF-β-mediated inhibition of T-cells in the context of tumor therapy, but to date no viable approaches for addressing this issue in T-cells for use in tumor therapy have been identified.

SUMMARY

[0008] In certain examples, provided herein are genome editing systems and related compositions and methods for the targeted editing of the nucleic acid sequence of TGFBR2. In certain examples, such targeted editing results in the alteration (e.g., down regulation) of TGFBR2 expression. In certain examples, such alteration of expression occurs in T cells. In certain examples, the alteration of TGFBR2 expression in T cells involves the use of a ribonucleoprotein (RNP) complex as a genome editing system comprising an RNA-guided nuclease protein complexed with a gRNA targeting the TGFBR2 gene. In certain examples, the alteration in TGFBR2 expression occurs as a result of a double-stranded break induced by the RNP and subsequent imperfect repair that leads to indels at and/or adjacent to the targeted TGFBR2 sequence.

[0009] In certain examples, the instant disclosure relates to genome editing systems that include a guide RNA with a targeting domain that is complementary to target sequence of a TGFBR2 gene and where the RNA-guided nuclease is a Cas9 nuclease. The targeting domain may be 70%, 80%, 85%, 90%, 95%, or 100% complementary.

[0009a] In one aspect, the present disclosure refers to a genome editing system comprising:

a gRNA comprising a targeting domain that is complementary with a target sequence of a Transforming Growth Factor β Receptor II (TGFBR2) gene; and

an RNA-guided nuclease.

[0010] In certain examples, the targeting domain has a length of 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 nucleotides.

[0011] In certain examples, the targeting domain has at least 18 contiguous nucleotides that are complementary to the TGFBR2 gene.

[0012] In certain examples, the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096. In certain examples, the targeting domain is configured to form a double strand break or a single strand break within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of the TGFBR2 target position.

[0013] In certain examples disclosed herein, the genome editing system is capable of altering TGFBR2 gene by knocking out the expression of the TGFBR2 gene or knocking down the expression of the TGFBR2 gene.

[0014] In certain examples, the genome editing systems disclosed herein incorporate a gRNA comprising a targeting domain configured to target a coding region or a non-coding region of the TGFBR2 gene, wherein said non-coding region comprises a promoter region, an enhancer region, an intron, the 3' UTR, the 5' UTR, or a polyadenylation signal region of said TGFBR2 gene; and the coding region comprises, e.g., an early coding region of said TGFBR2 gene.

[0015] In certain examples, the genome editing systems disclosed herein have a target sequence of the TGFBR2 gene comprising the sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3.

[0016] In certain examples, the genome editing systems disclosed herein have a target sequence of the TGFBR2 gene comprising the sequence selected from the group consisting of SEQ ID NOs: 4 to 10.

[0017] In certain examples, the genome editing systems disclosed herein incorporate a targeting domain comprising a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096. In certain examples, the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from the group consisting of: (a) SEQ ID NO: 5041; (b) SEQ ID NO: 5042; (c) SEQ ID NO: 5047; (d) SEQ ID NO: 5050; (e) SEQ ID NO: 5052; (f) SEQ ID NO: 5092; and (g) SEQ ID NO: 5093. In certain examples, the genome editing system incorporates pairs of gRNA molecules, including, e.g., gRNA pairs having target sequences SEQ ID NOS: 5042 and 5041, or 5042 and 5092, or SEQ ID NOS: 5042 and 5093, or SEQ ID NOS: 5093 and 5041.

[0018] In certain examples, the present disclosure relates to a composition comprising a gRNA molecule comprising a targeting domain that is complementary with a target sequence of a TGFBR2

gene. In certain examples, the composition comprises one, two, three, or four gRNA molecules. In certain examples, the composition further comprises an RNA-guided nuclease, e.g., a Cas9 molecule. In certain examples, the targeting domain incorporated into such compositions comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096. In certain examples, the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from the group consisting of: (a) SEQ ID NO: 5041; (b) SEQ ID NO: 5042; (c) SEQ ID NO: 5047; (d) SEQ ID NO: 5050; (e) SEQ ID NO: 5052; (f) SEQ ID NO: 5092; and (g) SEQ ID NO: 5093. In certain examples, the composition incorporates pairs of gRNA molecules, including, e.g., gRNA pairs having target sequences SEQ ID NOS: 5042 and 5041, or 5042 and 5092, or SEQ ID NOS: 5042 and 5093, or SEQ ID NOS: 5093 and 5041.

[0018a] In another aspect, the present disclosure refers to a composition comprising a gRNA comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene.

[0019] In certain examples, the present disclosure relates to a vector encoding a gRNA molecule comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene. In certain examples, the vector further encodes for an RNA-guided nuclease, e.g., a Cas9 molecule. In certain examples, the targeting domain of the gRNA encoded by the vector comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096. In certain examples, the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from the group consisting of: (a) SEQ ID NO: 5041; (b) SEQ ID NO: 5042; (c) SEQ ID NO: 5047; (d) SEQ ID NO: 5050; (e) SEQ ID NO: 5052; (f) SEQ ID NO: 5092; and (g) SEQ ID NO: 5093. In certain examples the vector is a viral vector. In certain examples, the vector is an adeno-associated virus (AAV) vector or a lentivirus (LV) vector.

[0019a] In another aspect, the present disclosure refers to a vector comprising a polynucleotide encoding a gRNA that comprises a targeting domain that is complementary with a target sequence of a TGFBR2 gene.

[0019b] In another aspect, the present disclosure refers to the vector as disclosed herein for use in reducing or eliminating said TGFBR2 gene expression in a cell, optionally wherein said cell is from a subject suffering from cancer.

[0020] In certain examples, the present disclosure is directed to a method of altering a TGFBR2 gene in a cell, comprising administering to said cell one of: (i) a genome editing system comprising a gRNA molecule comprising a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a Cas9 molecule; (ii) a vector comprising a polynucleotide encoding a gRNA molecule comprising a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a polynucleotide encoding a Cas9 molecule; or (iii) a composition comprising a gRNA molecule comprising a targeting domain that that is complementary with a target sequence of said TGFBR2 gene, and a Cas9 molecule.

[0020a] In another aspect, the present disclosure refers to an *ex vivo* method of altering expression of a TGFBR2 gene in a cell, comprising administering to said cell one of:

(i) a genome editing system comprising a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a RNA-guided nuclease; or

(ii) a vector comprising a polynucleotide encoding a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a polynucleotide encoding an RNA-guided nuclease.

[0020b] In another aspect, the present disclosure refers to use of one of:

(i) a genome editing system comprising a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a RNA-guided nuclease; or

(ii) a vector comprising a polynucleotide encoding a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a polynucleotide encoding an RNA-guided nuclease

in the manufacture of a medicament for altering expression of a TGFBR2 gene in a cell.

[0021] In certain examples, the present disclosure is directed to a cell comprising a genome editing system as described herein, a gRNA composition of as described herein, or a vector as described herein. In certain examples, the cell expresses TGFBR2. In certain examples, the cell is a T cell.

[0021a] In another aspect, the present disclosure refers to a cell comprising the genome editing system as disclosed herein, the composition as disclosed herein, or the vector as disclosed herein.

[0021b] In another aspect, the present disclosure refers to a cell altered as disclosed herein.

[0022] In certain examples, the present disclosure is directed to a gRNA and a RNA-guided

nuclease comprising a ribonucleoprotein (RNP) complex.

[0023] In certain examples, the present disclosure is directed to administering to a cell two or more RNP complexes comprising gRNAs with different targeting domains.

[0024] In certain examples, the present disclosure is directed to RNP complexes comprising enzymatically active Cas9 (eaCas9) nucleases.

[0025] In certain examples, the present disclosure is directed to RNP complexes comprising eaCas9 nucleases that form double strand breaks in a target nucleic acid or single strand breaks in a target nucleic acid.

[0026] In certain examples, the present disclosure is directed to two RNP complexes comprising distinct gRNAs are used to form offset single strand breaks in the TGFBR2 gene in a cell.

[0027] In certain examples, the present disclosure is directed to a cell that is a T cell or a Natural Killer (NK) cell. In certain examples, the cell further comprises an engineered T cell receptor (eTCR) or a chimeric antigen receptor (CAR).

[0028] In another aspect, the present disclosure refers to an RNA-guided nuclease-mediated method of altering TGFBR2 gene expression in a cell comprising: a) contacting the cell with a sufficient amount of a gRNA that targets TGFBR2 and an RNA-guided nuclease; and b) forming a first DNA double strand break near a TGFBR2 target position in a TGFBR2 gene of the cell, wherein the first DNA double strand break is repaired by NHEJ, wherein said repair alters the expression of the TGFBR2 gene.

[0029] In certain examples, the present disclosure is directed to forming a second DNA double strand break near the TGFBR2 target position. In certain examples, a first double strand break is formed within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of the TGFBR2 target position. In certain examples, the first and second double strand breaks are formed within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of the TGFBR2 target position. In certain examples, the first double strand break is formed in a coding region or a non-coding region of said TGFBR2 gene, wherein said non-coding region comprises a promoter region, an enhancer region, an intron, a 3' UTR, a 5' UTR, or a polyadenylation signal region of said TGFBR2 gene. In certain examples the first and second double strand breaks are formed in a coding region or a non-coding region of said TGFBR2 gene, wherein said non-coding region comprises a promoter region, an enhancer region, an intron, a 3' UTR, a 5' UTR, or a polyadenylation signal region of said TGFBR2 gene.

[0030] In certain examples, the coding region is selected from exon 3, exon 4, and exon 5.

[0031] In certain examples, the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of SEQ ID NOS: 5036 to 5096.

[0032] In certain examples, the RNA-guided nuclease is an S. pyogenes Cas9 nuclease, and said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of: (a) SEQ ID NO: 5041; (b) SEQ ID NO: 5042; (c) SEQ ID NO: 5047; (d) SEQ ID NO: 5050; (e) SEQ ID NO: 5052; (f) SEQ ID NO: 5092; and (g) SEQ ID NO: 5093.

[0033] In certain examples, the RNA-guided nuclease is an S. aureus Cas9 nuclease.

[0034] In certain examples, the RNA-guided nuclease is a mutant Cas9 nuclease.

[0035] In certain examples, the NHEJ repair produces an insertion or deletion with a frequency of greater than or equal to 20%.

[0036] In certain examples, the insertion or deletion frequency is greater than or equal to 30%, 40%, or 50%.

[0037] In another aspect, the present disclosure refers to a genome engineered cell comprising an insertion or deletion near or at a target position of a TGFBR2 gene, wherein said target position comprises a nucleotide sequence that is complementary to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096.

[0038] In certain examples, the insertion or deletion is within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of the TGFBR2 target position.

[0039] In certain examples, the cell is a T cell or NK cell. In certain examples, the cell further comprises a eTCR or CAR.

[0040] In another aspect, the present disclosure refers to a composition comprising: a) a population of genome engineered cells comprising an insertion or deletion near or at a target position of a TGFBR2 gene, wherein said target position comprises a nucleotide sequence that is complementary to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096; and b) a pharmaceutically acceptable buffer.

[0041] In certain examples, the population of cells comprises T cells or NK cells. In certain examples, the cell further comprises a eTCR or CAR.

[0042] In certain examples, the present disclosure is directed to a method of treating cancer in subject, comprising administering to the subject engineered immune cells, wherein the engineered immune cells have reduced expression of TGFBR2, and optionally expresses an engineered T Cell

Receptor (eTCR) or a Chimeric Antigen Receptor (CAR), wherein the engineered immune cells have an insertion or a deletion near or at a target position of the TGFBR2 gene.

[0042a] In another aspect, the present disclosure refers to use of engineered immune cells in the manufacture of a medicament for treating cancer in subject, wherein the engineered immune cells have reduced expression of TGFBR2, and optionally expresses an engineered T Cell Receptor (eTCR) or a Chimeric Antigen Receptor (CAR), wherein the engineered immune cells have an insertion or a deletion near or at a target position of the TGFBR2 gene.

[0043] In certain examples, the engineered immune cells comprise T cells or NK cells. In certain examples, the cell further comprises a eTCR or CAR.

[0044] In certain examples, the cancer is selected from the group consisting of: leukemia, lymphoma, e.g., chronic lymphocytic leukemia (CLL), acute-lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, acute myeloid leukemia, multiple myeloma, refractory follicular lymphoma, mantle cell lymphoma, indolent B cell lymphoma, B cell malignancies, cancers of the colon, lung, liver, breast, prostate, ovarian, skin, melanoma, bone, and brain cancer, ovarian cancer, epithelial cancers, renal cell carcinoma, pancreatic adenocarcinoma, Hodgkin's lymphoma, cervical carcinoma, colorectal cancer, glioblastoma, neuroblastoma, Ewing's sarcoma, medulloblastoma, osteosarcoma, synovial sarcoma, mesothelioma, and/or any cancer type that expresses TGF-β.

[0045] In certain examples, the T cells are CD4+ and/or CD8+ T cells.

[0046] In certain examples, the engineered immune cells maintain or have enhanced lysis activity against a target cancer cell relative to a non-engineered immune cell.

[0047] In certain examples, the engineered immune cells maintain or have increased expression of granzyme B and/or interferon gamma in the presence of TGFβ relative to non-engineered immune cells.

[0048] In certain examples, the engineered immune cells maintain or have improved persistence against repeated antigen stimulation relative to non-engineered immune cells.

[0049] In certain examples, the engineered immune cells maintain or have increased expression of CD25 relative to non-engineered immune cells.

[0050] In certain examples, the engineered immune cells maintain or have decreased expression of PD-1 relative to non-engineered immune cells.

[0051] In certain examples, the engineered immune cells maintain or have increased proliferation relative to non-engineered immune cells.

[0052] In another aspect, the present disclosure refers to a composition comprising a plurality of engineered T cells, wherein said engineered T cells exhibit reduced TGFBR2 gene expression relative to non-engineered T cells.

[0053] In certain examples, the engineered T cells exhibit a TGFBR2 gene expression level that is about 50%, about 40%, about 30%, about 20%, about 10% or about 5% the level of TGFBR2 expression in non-engineered T cells.

[0054] In certain examples, the engineered T cells further comprise expression of an eTCR or a

CAR.

[0055] In certain examples, the T cells are CD4+ T cells and/or CD8+ T cells.

[0056] In certain examples, the engineered T cells are further characterized by possessing: a) enhanced lysis activity against a target cancer cell relative to non-engineered T cells; b) maintained or increased expression of granzyme B and/or interferon gamma in the presence of TGFβ relative to non-engineered T cells; c) maintained or increased persistence against repeated antigen stimulation relative to non-engineered T cells; d) maintained or increased expression of CD25 relative to non-engineered T cells; e) maintained or decreased expression of PD-1 relative to non-engineered T cells; and/or f) maintained or increased proliferation relative to non-engineered T cells.

[0057] In another aspect, the present disclosure refers to a composition comprising a plurality of engineered T cells, wherein said engineered T cells exhibit reduced TGFBR2 gene expression relative to non-engineered T cells, said engineered T cells produced by contacting non-engineered T cells with a genome editing system comprising: a gRNA comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene; and an RNA-guided nuclease.

[0058] In certain examples, the engineered T cells are further transduced with a vector that expresses an eTCR or a CAR. In certain examples, the vector is a viral vector. In certain examples, the viral vector is an adeno-associated virus (AAV) vector or a lentivirus (LV) vector.

[0059] In certain examples, the RNA-guided nuclease is an S. pyogenes Cas9 nuclease, and said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of: (a) SEQ ID NO: 5041; (b) SEQ ID NO: 5042; (c) SEQ ID NO: 5047; (d) SEQ ID NO: 5050; (e) SEQ ID NO: 5052; (f) SEQ ID NO: 5092; and (g) SEQ ID NO: 5093.

[0060] In another aspect, the present disclosure refers to a composition comprising a plurality of engineered T cells, wherein said engineered T cells are deficient in TGFBR2 signaling.

[0061] In certain examples, the deficient TGFBR2 signaling is mediated by expressing a Dominant Negative (DN) form of the TGFBR2 in said engineered T cells.

[0062] In certain examples, the engineered T cells are further transduced with a vector that expresses an eTCR or a CAR. In certain examples, the vector is a viral vector. In certain examples, the viral vector is an adeno-associated virus (AAV) vector or a lentivirus (LV) vector.

[0063] In certain examples, the T cells are CD4+ T cells and/or CD8+ T cells.

[0064] In certain examples, the engineered T cells are further characterized by possessing: a) enhanced lysis activity against a target cancer cell relative to non-engineered T cells; b) maintained or increased expression of granzyme B and/or interferon gamma in the presence of TGFβ relative to non-

engineered T cells; c) maintained or increased persistence against repeated antigen stimulation relative to non-engineered T cells; d) maintained or increased expression of CD25 relative to non-engineered T cells; e) maintained or decreased expression of PD-1 relative to non-engineered T cells; and/or f) maintained or increased proliferation relative to non-engineered T cells.

[0065] In certain examples, the engineered immune cells further comprise reduced expression of wild-type TGFBR2.

[0066] In certain examples, the wild-type TGFBR2 expression is reduced by contacting the engineered immune cells with a genome editing system comprising: a gRNA comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene; and an RNA-guided nuclease.

[0067] In another aspect, the present disclosure refers to a ribonucleoprotein (RNP) complex comprising a gRNA that comprises a targeting domain that is complementary with a target sequence of a TGFBR2 gene and an RNA-guided nuclease.

[0068] In certain examples, the RNP of claim 117, wherein the RNA-guided nuclease is a Cas9 nuclease.

[0069] In certain examples, the RNP of claim 117, wherein the RNP is electroporated into cells.

[0070] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0071] Headings, including numeric and alphabetical headings and subheadings, are for organization and presentation and are not intended to be limiting.

[0072] Other features and advantages of the disclosure will be apparent from the detailed description, examples, drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] The accompanying drawings are intended to provide illustrative, and schematic rather than comprehensive, examples of certain aspects and embodiments of the present disclosure. The drawings are not intended to be limiting or binding to any particular theory or model, and are not necessarily to scale. Without limiting the foregoing, nucleic acids and polypeptides may be depicted as linear sequences, or as schematic two- or three-dimensional structures; these depictions are

CLAIMS

What is claimed is:

1. A genome editing system comprising:

a gRNA comprising a targeting domain that is complementary with a target sequence of a Transforming Growth Factor β Receptor II (TGFBR2) gene; and

an RNA-guided nuclease.

2. The genome editing system of claim 1, wherein:

the target sequence of the TGFBR2 gene comprises the sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3;

the target sequence of the TGFBR2 gene comprises the sequence selected from the group consisting of SEQ ID NOs: 4 to 10;

the targeting domain has at least 85% complementarity to the target sequence of the TGFBR2 gene;

the targeting domain is configured to form a double strand break or a single strand break within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of an TGFBR2 target position, thereby altering said TGFBR2 gene;

the TGFBR2 gene expression is knocked out or knocked down;

the gRNA targets a coding region or a non-coding region of said TGFBR2 gene, wherein the non-coding region comprises a promoter region, an enhancer region, an intron, a 3' UTR, a 5' UTR, or a polyadenylation signal region of said TGFBR2 gene, optionally wherein the coding region is selected from exon 3, exon 4, and exon 5;

the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of SEQ ID NOS: 5036 to 5096;

expression of TGFBR2 is reduced by 30% or more relative to a baseline measurement, optionally wherein expression of TGFBR2 protein is determined by Western blot or indirect intracellular staining flow cytometry; and/or

a frame-shift mutation is introduced into the TGFBR2 gene.

3. The genome editing system of any one of claims 1 or 2, wherein:

said RNA-guided nuclease is an *S. pyogenes* Cas9 nuclease, optionally wherein the *S. pyogenes* Cas9 nuclease recognizes a Protospacer Adjacent Motif (PAM) of NGG, and

said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than 3 nucleotides from, a nucleotide sequence selected from the group consisting of:

(a) SEQ ID NO: 5041;

(b) SEQ ID NO: 5042;

(c) SEQ ID NO: 5047;

(d) SEQ ID NO: 5050;

(e) SEQ ID NO: 5052;

(f) SEQ ID NO: 5092; and

(g) SEQ ID NO: 5093.

4. The genome editing system of any one of claims 1-3, wherein;

said RNA-guided nuclease is an *S. aureus* Cas9 nuclease, optionally wherein the *S. aureus* Cas9 nuclease recognizes a PAM of either NNNRRT or NNNRRV; and/or

the RNA-guided nuclease is a mutant Cas9 nuclease.

5. The genome editing system of any one of claims 1-4, wherein:

said gRNA is a modular gRNA or a chimeric gRNA;

the targeting domain has a length of about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 nucleotides;

the targeting domain comprises at least about 18 contiguous nucleotides that are complementary to the TGFBR2 gene;

the genome editing system comprises two, three or four gRNAs.

6. The genome editing system of claim 1, comprising:

at least one *S. pyogenes* Cas9 nuclease; and a gRNA comprising a combination of

SEQ ID NOS: 5042 and 5041, or a combination of 5042 and 5092, or a combination of SEQ ID NOS: 5042 and 5093, or a combination of SEQ ID NOS: 5093 and 5041.

7. The genome editing system of any one of claims 1-6 for use in altering said TGFBR2 gene in a cell, optionally wherein the cell is from a subject suffering from cancer.

8. A composition comprising a gRNA comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene.

9. The composition of claim 8, wherein:

the target sequence of the TGRBR2 gene comprises the sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3;

the target sequence of the TGRBR2 gene comprises the sequence selected from the group consisting of SEQ ID NOs: 4 to 10;

the targeting domain has at least 85% complementarity to the target sequence of the TGRBR2 gene;

the targeting domain has a length of about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 nucleotides;

the targeting domain comprises at least about 18 contiguous nucleotides that are complementary to the TGFBR2 gene;

the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of SEQ ID NOS: 5036 to 5096; and/or

the composition comprises one, two, three, or four gRNAs.

10. The composition of any one of claims 8 and 9, further comprising a Cas9 nuclease, optionally wherein:

the Cas9 nuclease is an *S. pyogenes* Cas9 nuclease or an *S. aureus* Cas9 nuclease, optionally wherein the *S. aureus* Cas9 nuclease recognizes a PAM of either NNNRRT or NNNRRV;

one or both of a wild-type Cas9 nuclease and a mutant Cas9 nuclease;

the Cas9 molecule is an *S. pyogenes* Cas9 molecule, optionally wherein the *S. pyogenes* Cas9 nuclease recognizes a Protospacer Adjacent Motif (PAM) of NGG and the targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from a nucleotide sequence selected from the group consisting of:

(a) SEQ ID NO: 5041;

(b) SEQ ID NO: 5042;

(c) SEQ ID NO: 5047;

(d) SEQ ID NO: 5050;

(e) SEQ ID NO: 5052;

(f) SEQ ID NO: 5092; and

(g) SEQ ID NO: 5093; and/or

the Cas9 nuclease comprises at least one *S. pyogenes* Cas9 nuclease; and a gRNA comprising a combination of SEQ ID NOS: 5042 and 5041, or a combination of 5042 and 5092, or a combination of SEQ ID NOS: 5042 and 5093, or a combination of SEQ ID NOS: 5093 and 5041.

11. The composition of any one of claims 8-10 for use in reducing or eliminating said TGFBR2 gene expression in a cell, optionally wherein said cell is from a subject suffering from cancer.

12. A vector comprising a polynucleotide encoding a gRNA that comprises a targeting domain that is complementary with a target sequence of a TGFBR2 gene.

13. The vector of claim 12, wherein said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of SEQ ID NOS: 5036 to 5096.

14. The vector of claim 12 or 13, further comprising a polynucleotide encoding a Cas9 nuclease. optionally wherein:

said Cas9 nuclease is an *S. pyogenes* Cas9 nuclease or an *S. aureus* Cas9 nuclease, optionally wherein said *S. aureus* Cas9 nuclease recognizes a PAM of either NNNRRT or

NNNRRV;

said Cas9 nuclease further comprises one or both of a wild-type Cas9 nuclease and a mutant Cas9 nuclease;

said Cas9 nuclease is an *S. pyogenes* Cas9 nuclease, optionally wherein said *S. pyogenes* Cas9 nuclease recognizes a Protospacer Adjacent Motif (PAM) of NGG, and said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of:

(a) SEQ ID NO: 5041;

(b) SEQ ID NO: 5042;

(c) SEQ ID NO: 5047;

(d) SEQ ID NO: 5050;

(e) SEQ ID NO: 5052;

(f) SEQ ID NO: 5092; and

(g) SEQ ID NO: 5093; and/or

the vector is a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) vector or a lentivirus (LV) vector.

15. The vector of any one of claims 12-14 for use in reducing or eliminating said TGFBR2 gene expression in a cell, optionally wherein said cell is from a subject suffering from cancer.

16. An *ex vivo* method of altering expression of a TGFBR2 gene in a cell, comprising administering to said cell one of:

(i) a genome editing system comprising a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a RNA-guided nuclease; or

(ii) a vector comprising a polynucleotide encoding a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a polynucleotide encoding an RNA-guided nuclease.

17. The method of claim 16, wherein:

said alteration comprises knockout of said TGFBR2 gene expression or knockdown of

said TGFBR2 gene expression; and/or

said cell is from a subject suffering from cancer.

18. The method of claim 16, wherein the gRNA and the RNA-guided nuclease comprise a ribonucleoprotein (RNP) complex.

19. The method of claim 18, comprising administering to the cell two or more RNP complexes comprising gRNAs with different targeting domains, optionally wherein two RNP complexes comprising distinct gRNAs are used to form offset single strand breaks in the TGFBR2 gene in the cell.

20. The method of claim 18, wherein the RNP complexes comprise enzymatically active Cas9 (eaCas9) nucleases, optionally wherein the RNP complexes comprise eaCas9 nucleases that form double strand breaks in a target nucleic acid or single strand breaks in a target nucleic acid.

21. A cell comprising the genome editing system of any one of claims 1-7, the composition of any one of claims 8-11, or the vector of any one of claims 12-15.

22. The cell of claim 21, wherein said cell expresses TGFBR2, optionally wherein said cell is a T cell or a Natural Killer (NK) cell, optionally further comprising an engineered T cell receptor (eTCR) or a chimeric antigen receptor (CAR).

23. A cell altered according to any one of claims 16-20.

24. An RNA-guided nuclease-mediated method of altering TGFBR2 gene expression in a cell comprising:

a) contacting the cell with a sufficient amount of a gRNA that targets TGFBR2 and an RNA-guided nuclease; and

b) forming a first DNA double strand break near a TGFBR2 target position in a TGFBR2 gene of the cell, wherein the first DNA double strand break is repaired by NHEJ, wherein said repair alters the expression of the TGFBR2 gene.

25. The method of claim 24, further comprising forming a second DNA double strand break near the TGFBR2 target position.

26. The method of claim 24, wherein:

the first double strand break is formed within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of the TGFBR2 target position; and/or

the first double strand break is formed in a coding region or a non-coding region of said TGFBR2 gene, wherein said non-coding region comprises a promoter region, an enhancer region, an intron, a 3' UTR, a 5' UTR, or a polyadenylation signal region of said TGFBR2 gene, optionally wherein said coding region is selected from exon 3, exon 4, and exon 5.

27. The method of claim 25, wherein:

the first and second double strand breaks are formed within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about 25bp, or about 10bp of the TGFBR2 target position; and/or

the first and second double strand breaks are formed in a coding region or a non-coding region of said TGFBR2 gene, wherein said non-coding region comprises a promoter region, an enhancer region, an intron, a 3' UTR, a 5' UTR, or a polyadenylation signal region of said TGFBR2 gene.

28. The method of any one of claims 25-27, wherein said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of SEQ ID NOS: 5036 to 5096.

29. The method of any one of claims 25-28, wherein said RNA-guided nuclease is an *S. pyogenes* Cas9 nuclease, and said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of:
(a) SEQ ID NO: 5041;
(b) SEQ ID NO: 5042;
(c) SEQ ID NO: 5047;
(d) SEQ ID NO: 5050;
(e) SEQ ID NO: 5052;
(f) SEQ ID NO: 5092; and
(g) SEQ ID NO: 5093.

30. The method of any one of claims 24-28, wherein:

said RNA-guided nuclease is an *S. aureus* Cas9 nuclease; and/or

wherein said RNA-guided nuclease is a mutant Cas9 nuclease.

31. The method of claim 24, wherein the NHEJ repair produces an insertion or deletion with a frequency of greater than or equal to 20%.

32. The method of claim 31, wherein the insertion or deletion frequency is greater than or equal to 30%, 40%, or 50%.

33. A genome engineered cell comprising an insertion or deletion near or at a target position of a TGFBR2 gene, wherein said target position comprises a nucleotide sequence that is complementary to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096.

34. The cell of claim 33, wherein:

the insertion or deletion is within about 500bp, about 450bp, about 400bp, about 350bp, about 300bp, about 250bp, about 200bp, about 150bp, about 100bp, about 50bp, about

25bp, or about 10bp of the TGFBR2 target position; and/or

the cell is a T cell or NK cell, optionally further comprising a eTCR or CAR.

35. A composition comprising:

a. a population of genome engineered cells comprising an insertion or deletion near or at a target position of a TGFBR2 gene, wherein said target position comprises a nucleotide sequence that is complementary to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from SEQ ID NOS: 5036 to 5096; and

b. a pharmaceutically acceptable buffer.

36. The composition of claim 35, wherein the population of cells comprises T cells or NK cells, optionally wherein the T cells or NK cells further comprise a eTCR or a CAR.

37. Use of engineered immune cells in the manufacture of a medicament for treating cancer in subject, wherein the engineered immune cells have reduced expression of TGFBR2, and optionally expresses an engineered T Cell Receptor (eTCR) or a Chimeric Antigen Receptor (CAR), wherein the engineered immune cells have an insertion or a deletion near or at a target position of the TGFBR2 gene.

38. The use of claim 37, wherein the engineered immune cells comprise T cells or NK cells, optionally wherein:

the eTCR or CAR has antigen specificity to a cancer cell; and/or

the T cells are CD4+ and/or CD8+ T cells.

39. The use of claim 37, wherein the cancer is selected from the group consisting of: leukemia, lymphoma, chronic lymphocytic leukemia (CLL), acute-lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, acute myeloid leukemia, multiple myeloma, refractory follicular lymphoma, mantle cell lymphoma, indolent B cell lymphoma, B cell malignancies,

cancers of the colon, lung, liver, breast, prostate, ovarian, skin, melanoma, bone, and brain cancer, ovarian cancer, epithelial cancers, renal cell carcinoma, pancreatic adenocarcinoma, Hodgkin's lymphoma, cervical carcinoma, colorectal cancer, glioblastoma, neuroblastoma, Ewing's sarcoma, medulloblastoma, osteosarcoma, synovial sarcoma, mesothelioma, and/or any cancer type that expresses TGF-β.

40. The use of claim 37, wherein:

the engineered immune cells maintain or have enhanced lysis activity against a target cancer cell relative to a non-engineered immune cell;

the engineered immune cells maintain or have increased expression of granzyme B and/or interferon gamma in the presence of TGFβ relative to non-engineered immune cells;

the engineered immune cells maintain or have improved persistence against repeated antigen stimulation relative to non-engineered immune cells;

the engineered immune cells maintain or have increased expression of CD25 relative to non-engineered immune cells;

the engineered immune cells maintain or have decreased expression of PD-1 relative to non-engineered immune cells; and/or

the engineered immune cells maintain or have increased proliferation relative to non-engineered immune cells.

41. A composition comprising a plurality of engineered T cells, wherein said engineered T cells exhibit reduced TGFBR2 gene expression relative to non-engineered T cells.

42. The composition of claim 98, wherein:

the engineered T cells exhibit a TGFBR2 gene expression level that is about 50%, about 40%, about 30%, about 20%, about 10% or about 5% the level of TGFBR2 expression in non-engineered T cells;

the engineered T cells further comprise expression of an eTCR or a CAR; and/or

the T cells are CD4+ T cells and/or CD8+ T cells.

43. The composition of claim 41, wherein the engineered T cells are further characterized by possessing:

a) enhanced lysis activity against a target cancer cell relative to non-engineered T cells;

b) maintained or increased expression of granzyme B and/or interferon gamma in the presence of TGFβ relative to non-engineered T cells;

c) maintained or increased persistence against repeated antigen stimulation relative to non-engineered T cells;

d) maintained or increased expression of CD25 relative to non-engineered T cells;

e) maintained or decreased expression of PD-1 relative to non-engineered T cells; and/or

f) maintained or increased proliferation relative to non-engineered T cells.

44. A composition comprising a plurality of engineered T cells, wherein said engineered T cells exhibit reduced TGFBR2 gene expression relative to non-engineered T cells, said engineered T cells produced by contacting non-engineered T cells with a genome editing system comprising:

a gRNA comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene; and

an RNA-guided nuclease.

45. The composition of claim 44, wherein the engineered T cells are further transduced with a vector that expresses an eTCR or a CAR.

46. The composition of claim 45, wherein the vector is a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) vector or a lentivirus (LV) vector.

47. The composition of claim 45, wherein said RNA-guided nuclease is an *S. pyogenes* Cas9

nuclease, and said targeting domain comprises a nucleotide sequence that is identical to, or differs by no more than about 3 nucleotides from, a nucleotide sequence selected from the group consisting of:

(a) SEQ ID NO: 5041;

(b) SEQ ID NO: 5042;

(c) SEQ ID NO: 5047;

(d) SEQ ID NO: 5050;

(e) SEQ ID NO: 5052;

(f) SEQ ID NO: 5092; and

(g) SEQ ID NO: 5093.

48. A composition comprising a plurality of engineered T cells, wherein said engineered T cells are deficient in TGFBR2 signaling.

49. The composition of claim 48, wherein deficient TGFBR2 signaling is mediated by expressing a Dominant Negative (DN) form of the TGFBR2 in said engineered T cells.

50. The composition of claim 49, wherein the engineered T cells are further transduced with a vector that expresses an eTCR or a CAR, optionally wherein the T cells are CD4+ T cells and/or CD8+ T cells.

51. The composition of claim 50, wherein the vector is a viral vector, optionally wherein the viral vector is an adeno-associated virus (AAV) vector or a lentivirus (LV) vector.

52. The composition of claim 48, wherein the engineered T cells are further characterized by possessing:

a) enhanced lysis activity against a target cancer cell relative to non-engineered T cells;

b) maintained or increased expression of granzyme B and/or interferon gamma in the presence of TGFβ relative to non-engineered T cells;

c) maintained or increased persistence against repeated antigen stimulation relative to non-engineered T cells;

d) maintained or increased expression of CD25 relative to non-engineered T cells;

e) maintained or decreased expression of PD-1 relative to non-engineered T cells; and/or

f) maintained or increased proliferation relative to non-engineered T cells.

53. The composition of claim 48, wherein the engineered immune cells further comprise reduced expression of wild-type TGFBR2.

54. The composition of claim 53, wherein wild-type TGFBR2 expression is reduced by contacting the engineered immune cells with a genome editing system comprising:

a gRNA comprising a targeting domain that is complementary with a target sequence of a TGFBR2 gene; and

an RNA-guided nuclease.

55. A ribonucleoprotein (RNP) complex comprising a gRNA that comprises a targeting domain that is complementary with a target sequence of a TGFBR2 gene and an RNA-guided nuclease.

56. The RNP of claim 55, wherein the RNA-guided nuclease is a Cas9 nuclease.

57. The RNP of claim 55, wherein the RNP is electroporated into cells.

58. Use of one of:

(i) a genome editing system comprising a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a RNA-guided nuclease; or

(ii) a vector comprising a polynucleotide encoding a gRNA that comprises a targeting domain that is complementary with a target sequence of said TGFBR2 gene, and a

polynucleotide encoding an RNA-guided nuclease

in the manufacture of a medicament for altering expression of a TGFBR2 gene in a cell.